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Effect of Apoplastic Solutes on Water Potential in Elongating Sugarcane Leaves[1]

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FREDERICK C. MEINZER* AND PAUL H. MOORE
Hawaiian Sugar Planters' Association (F.C.M.) *and United States Department of Agriculture/Agricultural Research Service* (P.H.M.), *Aiea, Hawaii 96701*

ABSTRACT

Solute concentration in the apoplast of growing sugarcane (*Saccharum* spp. hybrid) leaves was measured using one direct and several indirect methods. The osmotic potential of apoplast solution collected directly by centrifugation of noninfiltrated tissue segments ranged from −0.25 megapascal in mature tissue to −0.35 megapascal in tissue just outside the elongation zone. The presence of these solutes in the apoplast manifested itself as a tissue water potential equal to the apoplast osmotic potential. Since the tissue was not elongating, the measurements were not influenced by growth-induced water uptake and no significant tension was detected with the pressure chamber. Further evidence for a significant apoplast solute concentration was obtained from pressure exudation experiments and comparison of methods for estimating tissue apoplast water fraction. For elongating leaf tissue the centrifugation method could not be used to obtain direct measurements of apoplast solute concentration. However, several other observations suggested that the apoplast water potential of −0.35 to −0.45 megapascal in elongating tissue had a significant osmotic component and small, but significant tension component. Results of experiments in which exudate was collected from pressurized tissue segments of different ages suggested that a tissue age-dependent dynamic equilibrium existed between intra- and extracellular solutes.

The characteristics of solute and water partitioning between the symplast and apoplast of plant tissues have important implications for studies of plant-water relations. Interpretation of pressure chamber and thermocouple psychrometer measurements often relies on the tacit assumptions that osmotic potential and relative volume of water in the apoplastic space are negligible. While the invalidity of either or both of these assumptions has widely recognized consequences, relatively few studies have dealt quantitatively with the influence of apoplastic solutes and water. Values of $\Psi\pi a$[2] reported for leaf and stem tissue range from −0.03 to −0.3 MPa in a number of species (2, 7, 12, 13, 18). However, at present it is not clear how much of this order of magnitude range is due to differences in methodological approach and interpretation, since the extremes represent values reported for growing pea stem tissue by different groups of investigators (7, 18). There appear to be no reports available of direct measurements of $\Psi\pi a$ in grass leaves.

The influence of $\Psi\pi a$ on bulk tissue Ψ would be greatest in nontranspiring tissues at or near full hydration. If internal Ψ equilibrium prevails under these conditions, then symplast and apoplast Ψ would be equal and $\Psi\pi a$ would largely determine bulk tissue Ψ. Under drier conditions small transpiration-induced declines in tissue water content would diminish the influence of $\Psi\pi a$, and bulk tissue Ψ would reflect the significant tension generated in the xylem and cell wall space water. Knowledge of the magnitude of $\Psi\pi a$ is particularly crucial for expanding leaf and stem tissue in view of two distinct hypotheses proposed to explain the means by which water supply can limit growth. The discrepancy between the two hypotheses is centered on the interpretation of the origin of the lowered or 'growth-induced' water potential observed in expanding leaf and stem tissue of several species (3, 4, 16, 23). From measurements of apoplast solute concentration and hydraulic conductivity in pea stems, Cosgrove and Cleland (8) have concluded that hydraulic conductance does not limit the rate of cell enlargement and that the growth-induced Ψ is due largely to the presence of apoplastic solutes. Others, however, have found that growth-induced Ψ for pea stems and other tissues arises from hydraulic limitations on water uptake during growth and not the presence of apoplastic solutes (3, 18).

With the exception of maize leaves (15, 22, 23), little information is available concerning the water relations of expanding monocotyledonous leaves which differ substantially in their development patterns from dicotyledonous leaves (1). In this study we have employed a number of pressure chamber and psychrometric techniques to determine profiles of solute and water partitioning between the symplast and apoplast along expanding sugarcane leaves. This was undertaken as part of a broader study aimed at characterizing the basic water relations of expanding sugarcane leaves with particular regard to understanding the events associated with cessation of elongation when water is limiting.

MATERIALS AND METHODS

Plant Material. Tissue from greenhouse-grown sugarcane (*Saccharum* spp. hybrid) cv H65-7052 was used for all measurements. Plants were grown from lateral buds on stem segments sown in soil-filled pots and watered daily. In order to ensure full hydration, tissue samples were excised prior to sunrise when guttation water was usually present on leaf margins. Sample preparation and manipulation were always carried out in a humidified chamber.

The sugarcane leaf spindle is a cylinder of several concentric whorls of tightly packed elongating leaves positioned above the shoot apical meristem (Fig. 1). Its relatively large size (~1 cm diameter) makes it well-suited for experimental manipulation, either as a unit or as individual leaves of different ages, but elongating at the same rate. The youngest leaf whose blade is fully exposed and not enclosed in the sheaths of older leaves is

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[2] Abbreviations: $\Psi\pi a$: apoplast osmotic potential; Ψ: water potential; TVD: top visible dewlap; $\Psi\pi$: osmotic potential; $\Psi\pi b$ bulk tissue osmotic potential; $\Psi\pi s$: symplast osmotic potential: R_a: apoplast water fraction; Ψp: turgor pressure.

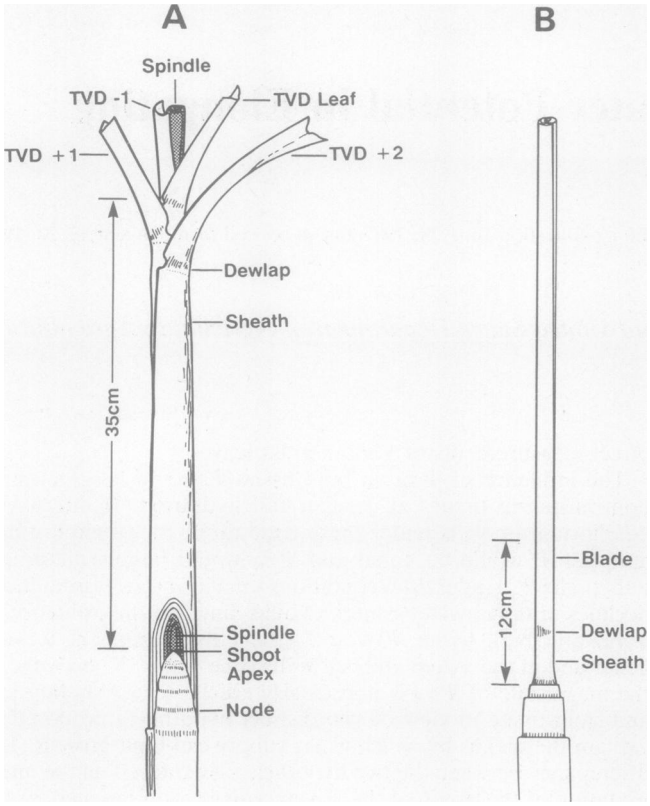

FIG. 1. A, Apical portion of sugarcane plant showing the elongating leaf spindle enclosed within the sheaths of the older leaves; B, older leaf sheaths cut away to show the leaf spindle containing the TVD − 2 and younger leaves.

designated as the TVD leaf. This leaf is also the youngest leaf with no significant blade or sheath elongation. Successively younger leaves are designated TVD minus 1 (TVD − 1), TVD − 2, . . . TVD − n. The leaf blade and sheath elongation zone was delineated by piercing shoots with a hypodermic needle above the apex at measured intervals and then excising and dissecting the shoot 24 h later to remeasure the intervals between the puncture marks for TVD through TVD − 4 leaves. Elongation rates were synchronous beginning with the TVD − 2 leaf which was experiencing both blade and sheath elongation. The younger spindle leaves contained only blade tissue.

Apoplast Osmotic Potential ($\Psi\pi a$). Segments of leaf spindle containing the TVD − 2 and younger leaves were used. Measurements obtained with one direct and two indirect methods were compared. In the direct method small samples of apoplastic solution were extracted from noninfiltrated spindle segments by centrifugation. Ends of freshly excised segments were dipped for 1 to 2 s in distilled water, blotted dry, then placed upright in the barrel of a polyethylene syringe with the lower cut end resting on two filter paper disks. The syringes were stoppered to prevent tissue dehydration and centrifuged at 120 g for 20 min. The saturated disks were then removed, immediately wrapped in aluminum foil, and frozen at −20°C until determination of osmotic potential in a vapor pressure osmometer (Wescor 5100B, Logan, UT).

In a second method, total Ψ of living spindle segments was measured psychrometrically in order to evaluate the hypothesis that Ψ would equal $\Psi\pi a$ under conditions of Ψ equilibrium within the tissue and zero tension in the apoplastic space. Spindle segments 5 mm thick were sealed in psychrometer chambers (Wescor C-52) and Ψ was measured with a microvoltmeter (Wescor HR-33T) in the dew point mode at frequent intervals throughout an incubation period of several hours.

In a third method, 10 cm spindle segments were inserted through a rubber stopper and sealed in a pressure chamber lined with moist filter paper. A rubber balloon diaphragm was sealed to the rubber stopper to prevent dehydration from passage of gas through the sample. The exposed spindle end was enclosed in a humidified chamber consisting of an inverted beaker lined with moist filter paper. Exudate from the exposed end was collected on filter paper disks as pressure was increased in 0.1 to 0.2 MPa increments. Each pressure was held for 3 min after which the filter paper was removed and the tissue end blotted to remove excess exudate before going to the next pressure. The time required for completion of exudation varied with tissue age and pressure applied, but 3 min at each pressure were always sufficient to obtain several saturated filter paper disks. These were wrapped in foil and stored in vials at −20°C pending osmotic potential determination.

Bulk Tissue and Symplast $\Psi\pi$. Excised shoots were dissected in order to obtain cylinders of spindle containing leaves whose elongation rates were synchronous (TVD − 2 and younger). These cylinders were cut transversely at predetermined distances with respect to the shoot apical meristem. The sections were transferred to preweighed vials and frozen immediately at −70°C. The vials were weighed upon thawing and then the tissue was rapidly macerated to obtain a sample of sap for determination of osmotic potential with the vapor pressure osmometer. The samples were then oven-dried at 70°C for dry weight determination. This procedure permitted calculation of bulk tissue solute content (mOsmol g^{-1} dry weight) in addition to $\Psi\pi$.

Symplast $\Psi\pi$ and solute content were measured for individual leaf segments with the pressure-volume method. The TVD − 2 leaf was divided into 12 cm segments beginning at the shoot apex. Sections of lamina were removed from one side of the midrib, wrapped in preweighed aluminum foil sheaths, and weighed to determine turgid weight. The basal end of the leaf blade was then extended from the foil sheath and inserted through a slit in a rubber stopper. A rubber balloon was placed over the portion of the leaf blade facing the inside of the pressure chamber and sealed to the rubber stopper with a nylon cable tie. The stopper assemblies were then sealed inside a specially constructed five-chamber apparatus lined with moist filter paper. Sap collected from the exposed leaf blade ends was collected in preweighed, tissue paper-filled vials kept in contact with the cut surface. In order to retard evaporation from the tissue paper between weighings, an inverted beaker lined with moist filter paper was placed over the vial and leaf blade end. Chamber pressure was successively raised in 0.1 to 0.2 MPa increments with each new pressure being held for 15 min. Data were plotted as the reciprocal of balance pressure on the ordinate *versus* relative water deficit. Values of osmotic potential at full turgor were obtained by extrapolation of the linear portion of the plot to the ordinate. Symplastic solute content (mOsmol g^{-1} dry weight) was calculated according to Tyree *et al.* (20).

Relative Apoplast Water Volume. Three different methods were used to measure leaf tissue R_a and to provide indirect evidence concerning the magnitude of apoplast solute concentration. In the first method

$$R_a = 1 - \frac{\Psi\pi b}{\Psi\pi s} \quad (1)$$

where $\Psi\pi b$ is bulk tissue $\Psi\pi$ measured with the osmometer and $\Psi\pi s$ is full turgor symplastic $\Psi\pi$ calculated from pressure-volume curves. In this method it is assumed that symplastic solutes exhibit ideal behavior upon dilution by apoplastic water and that the apoplastic water is free of solutes in the living tissue. In the second method, the linear portion of the pressure-volume curve was extrapolated to the abscissa (relative water deficit) at $1/\Psi = 0$ to obtain an estimate of symplastic water fraction. This was

then subtracted from one to obtain R_a. In the third method, $\Psi\pi b$ was assumed to be a weighted average of $\Psi\pi s$ and $\Psi\pi a$ according to the relationship

$$\Psi\pi a \cdot R_a + \Psi\pi s\,(1-R_a) = \Psi\pi b \qquad (2)$$

where $\Psi\pi a$ is the $\Psi\pi$ of the apoplastic solution extracted by centrifugation. Solving for R_a yields:

$$R_a = \frac{\Psi\pi b - \Psi\pi s}{\Psi\pi a - \Psi\pi s} \qquad (3)$$

Solute Identification. Apoplast solution collected by centrifugation was assayed for reducing sugars by the copper sulfate-arsenomolybdate method (17) and sucrose by the anthrone method (21). Potassium concentration in diluted apoplast solution was determined with a K^+ electrode (93-19 Orion Research, Cambridge, MA). Organic acids were assayed by HPLC with variable wavelength UV detection.

RESULTS

Bulk Tissue Ψ and $\Psi\pi$ Profiles. The zone of most rapid elongation for the TVD − 2 and younger leaves was located approximately 5 cm from the shoot apex, but the total length of the expanding zone was about 12 cm (Fig. 2). Bulk leaf Ψ determined psychrometrically ranged from −0.42 MPa in the zone of most rapid elongation to about −0.25 MPa in nonelongating tissue at 35 cm from the shoot apex. In contrast to Ψ, bulk leaf $\Psi\pi$ was relatively constant in both expanding and nonexpanding portions of the leaf. Between the zone of maximum elongation and the end of the elongation zone there was a small but consistent increase in $\Psi\pi$ averaging 0.07 MPa.

The Ψ of excised nonelongating leaf spindle segments increased during an incubation period of several hours in the psychrometer chamber (Fig. 3). If, after several hours psychrometer chambers were briefly opened, ventilated and resealed, Ψ returned to its previous value within about 20 min for the youngest tissue and 40 min for the most mature tissue. This confirmed that the apparent changes in Ψ observed over several hours were real and not the result of slow vapor equilibration. In order to standardize measurements, values taken after the initial 30 min in the chamber were used in Figure 2. Segments cut from the most rapidly elongating portion of the spindle, on the other hand,

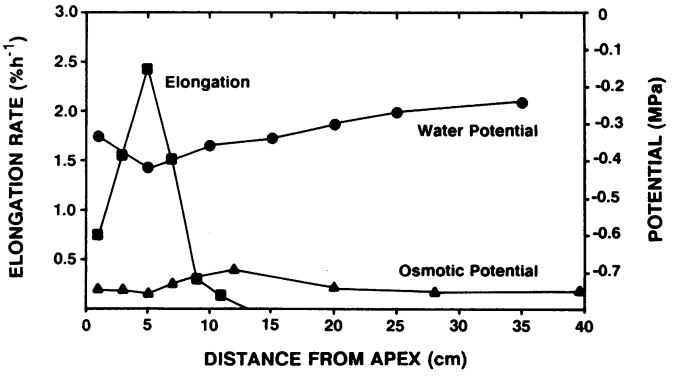

FIG. 2. Elongation rate and bulk tissue water and osmotic potential of sugarcane leaves in relation to distance from the shoot apical meristem. Measurements were taken from leaf spindle containing the TVD − 2 and younger leaves. Water potential measurements were obtained from 0.5 cm long cylinders of leaf spindle after 30 min equilibration in a psychrometer chamber (see text and Fig. 2). Sap expressed from 2 cm long frozen and thawed leaf spindle segments was used for osmotic potential determinations. Values are means of five samples. SEs range from 0.05 to 0.20% h^{-1} for elongation and 0.02 to 0.04 MPa for water and osmotic potential.

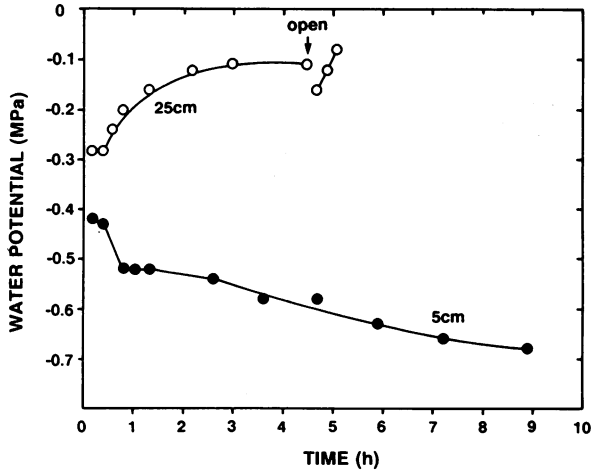

FIG. 3. Water potential of sugarcane leaf spindle segments excised at 5 cm and 25 cm from the shoot apex. Each plot is a representative time course for a single segment. In one case the psychrometer chamber was briefly opened and resealed at the time indicated by the arrow.

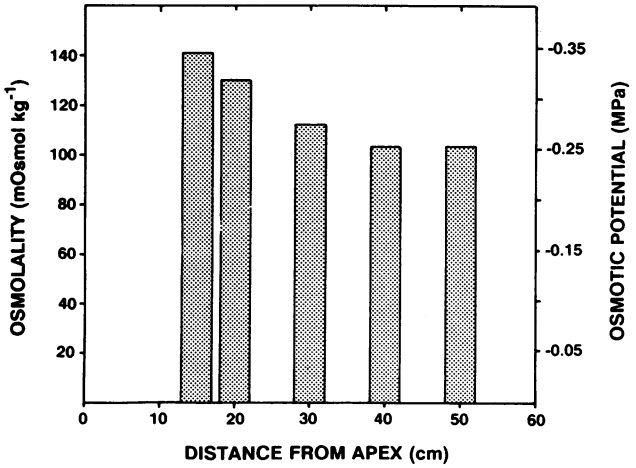

FIG. 4. Osmolality and osmotic potential of apoplast solution extracted from sugarcane leaf spindle segments by centrifugation. Segments 3.5 cm long were cut at different distances from the shoot apex. Values are means of five segments with SEs ranging from 6 to 12 mOsmol kg^{-1}.

exhibited a decline in Ψ during a holding period of several hours in the psychrometer chamber (Fig. 3). The average Ψ for segments cut at 5 cm from the shoot apex declined 0.25 MPa from −0.45 MPa at 30 min to −0.70 MPa after 7 to 9 h in the psychrometer chamber. This was probably attributable to a decrease in turgor due to continued stress relaxation of cell walls after excision.

Apoplast Osmotic Potential ($\Psi\pi a$). The osmotic potential of solution extracted by centrifugation of leaf segments ranged from −0.35 MPa at 15 cm from the shoot apex to −0.25 MPa in nearly mature tissue at 50 cm from the shoot apex (Fig. 4). At comparable distances from the shoot apex, $\Psi\pi$ for extracted apoplast solution was generally within 0.03 MPa of bulk tissue Ψ determined psychrometrically (*cf.* Figs. 2 and 4). Using this method even fully mature TVD leaf tissue yielded $\Psi\pi a$ values between −0.2 and −0.3 MPa (data not shown). The volume of solution extracted from a 2 cm tissue segment averaged about 8 μl. Two criteria were used to detect leakage of solutes from damaged cells. Discoloration of the solution due to release of oxidases and a $\Psi\pi$ approaching that of the bulk tissue indicated

that segments nearer than 15 cm to the shoot apex were damaged by centrifugation. The $\Psi\pi$ of solution collected from them is not shown in Figure 4. Centrifugation at reduced speeds to avoid tissue damage did not yield enough solution to fully saturate the filter paper disks.

When spindle segments were pressurized to obtain exudate for $\Psi\pi$ determination, the osmolality of the exudate at first decreased sharply with increasing pressure, then stabilized at higher applied pressures (Fig. 5A). The osmolality of exudate obtained at all pressures was highest in the younger segments closest to the shoot apex. Exudate obtained at the lowest pressure in these experiments probably contained a mixture of apoplastic solutes and solutes released from cells damaged by excision. The drop in osmolality at higher pressures reflected the increasing contribution of membrane-filtered symplastic water to the exudate volume. It was therefore not possible to obtain direct measurements of $\Psi\pi a$ from these experiments. However, the osmolality of the exudate obtained at lower pressures was in general agreement with that determined for apoplast solution extracted by centrifugation (*cf.* Figs. 4 and 5).

On several occasions 10 cm segments 10 to 20 cm from the shoot apex were excised prior to sunrise, sealed in plastic bags, and held for 24 h prior to subjecting them to the pressure exudation treatment. In an intact leaf spindle the zone of tissue 10 to 20 cm from the shoot apex would have been located at about 13 to 23 cm from the apex after 24 h. Osmolality *versus* pressure profiles for these incubated segments were comparable to those for nonincubated segments 20 to 30 cm from the shoot apex (Fig. 5A). Thus, tissue maturation in terms of changes in pressure-induced solute efflux appeared to continue normally even in excised tissue.

In other experiments the course of pressure exudation was interrupted by holding segments for 30 min at 0.5 MPa after exposure to 0.8 MPa for 3 min. When the pressure was again raised to 0.8 MPa the osmolality of the resulting exudate was higher than that previously collected at 0.8 MPa (Fig. 5B). This and the subsequent concentration profile at higher pressures suggested that a continued and possibly regulated release of solutes to the apoplast had occurred. Concentration of the exudate due to evaporation seemed unlikely since the exposed end was enclosed in a humid chamber. Removal of the initial exudate that appeared after return to 0.8 MPa further reduced any influence of evaporation at the cut surface.

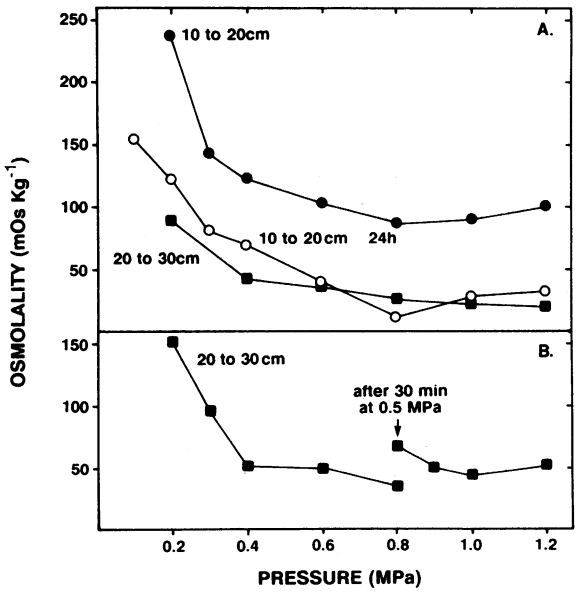

FIG. 5. A, Osmolality of exudate from the cut surface of pressurized sugarcane leaf spindle segments. Each pressure was held for 3 min. Plots are means of five 10 cm long segments cut between 10 and 20 cm and 20 and 30 cm from the shoot apex. Some segments were held 24 h in plastic bags prior to being pressurized (open circles). SEs range from 22 mOsmol kg^{-1} at lower pressures to 2 mOsmol kg^{-1} at higher pressures. B, Osmolality of exudate from a pressurized segment showing the effect of interrupting exudation for 30 min by lowering the pressure to 0.5 MPa after pressurization at 0.8 MPa for 3 min. Points represent samples collected during a 3 min holding period at each pressure.

Apoplast Water Fraction. Values of R_a determined by three methods for the TVD − 2 and TVD leaves indicated that a substantial fraction of the leaf water was located in the apoplast (Table I). The magnitude of R_a was sufficient to warrant its consideration in correcting bulk tissue $\Psi\pi$ determined from frozen and thawed tissue to obtain symplastic $\Psi\pi$. Agreement between estimates of R_a obtained from pressure-volume curves and Eq. 3 was generally close with the exception of the youngest segment of the TVD − 2 leaf examined. Data for the leaf segment 0 to 12 cm from the shoot apex were not included because the delicate elongating tissue gave inconsistent results when subjected to the pressure-volume procedure. Dividing $\Psi\pi b$ by $\Psi\pi s$ consistently gave lower estimates of R_a than the other two methods because $\Psi\pi b$ reflected mixing of symplastic contents with apoplastic water containing solutes rather than solute-free water. Considering the length of the extrapolation involved in determining R_a from pressure-volume curves and the effect of small changes in the slope of the regression line, the most reliable method for determining R_a was probably Eq. 3. In Eq. 3 determination of $\Psi\pi s$ involved a relatively short extrapolation and determination of $\Psi\pi b$ and $\Psi\pi a$ was direct.

Symplast $\Psi\pi$ and Leaf Turgor. Full turgor symplast $\Psi\pi$ determined from pressure-volume curves remained relatively constant along a tissue age gradient (Table II). Patterns of $\Psi\pi s$ and $\Psi\pi b$ in relation to tissue age were thus similar except that $\Psi\pi b$ was higher than $\Psi\pi s$ due to mixing of symplastic contents with apoplastic solution upon thawing of frozen tissue. Use of $\Psi\pi b$ rather than $\Psi\pi s$ to calculate bulk tissue turgor (Ψp) caused it to be underestimated by 0.15 to 0.2 MPa when bulk tissue Ψ was determined psychrometrically (Table II). Subtraction of $\Psi\pi s$ from pressure chamber measurement of bulk tissue Ψ overestimated $\Psi\pi$ because balancing pressures were invariably less than 0.02 MPa (data not shown) and did not reflect the presence of apoplastic solutes as did Ψ determined psychrometrically.

Identity of Apoplast Solutes. Analysis of apoplastic solution

Table I. *Comparison of Methods for Determination of Apoplastic Water Fraction in Sugarcane Leaves*

In the first method, R_a was calculated as $1\text{-}\Psi\pi b/\Psi\pi s$ assuming that symplastic solutes exhibited ideal behavior upon dilution by apoplastic water and that the apoplastic water was free of solutes in the living tissue. In the second method, the linear portion of the tissue pressure-volume curve was extrapolated to the abscissa (relative water deficit) at $1/\Psi = 0$. The resulting estimate of tissue symplastic water fraction was subtracted from one to obtain R_a. In the third method, $\Psi\pi b$ was assumed to be a weighted average of $\Psi\pi s$ and $\Psi\pi a$ according to the relationship $\Psi\pi b = \Psi\pi a \cdot R_a + \Psi\pi s(1 - R_a)$. Solving for R_a yielded $R_a = (\Psi\pi b - \Psi\pi s)/(\Psi\pi a\text{-}\Psi\pi s)$

Leaf Tissue	Apoplastic Water Fraction Method		
	1	2	3
12–24 cm from apex	0.17	0.34	0.26
24–36 cm from apex	0.19	0.28	0.27
36–48 cm from apex	0.21	0.31	0.29
TVD Leaf	0.26	0.33	0.35

Table II. *Water Potential Components of Sugarcane Leaves as a Function of Distance From the Shoot Apex*

Tissue Ψ was determined with a thermocouple psychrometer. Values represent averages for three 5 mm thick spindle segments cut from the ends and center of each 12 cm segment. Symplast $\Psi\pi$ was calculated by extrapolating the linear portion of the tissue pressure-volume curve to the ordinate ($1/\Psi$). Bulk tissue $\Psi\pi$ was determined in a vapor pressure osmometer for sap expressed from previously frozen tissue. All values are means of five samples; SE ranged from 0.02 to 0.03 MPa.

Distance from Apex	$\Psi\pi$		Ψ	Ψp	
	Symplast	Bulk tissue		$\Psi - \Psi\pi s$	$\Psi - \Psi\pi b$
cm			*MPa*		
0–12		−0.73	−0.42	0.50[a]	0.31
12–24	−0.90	−0.75	−0.30	0.60	0.45
24–36	−0.94	−0.75	−0.26	0.68	0.49
36–48	−0.91	−0.70	−0.22	0.69	0.48

[a] Estimated from Ψ at 0 to 12 cm minus $\Psi\pi s$ for 12 to 48 cm.

collected by centrifugation showed that about 13% of its osmolality was attributable to reducing sugars and sucrose and another 18% to K^+ (Table III). Aconitic acid, a major organic acid in sugarcane leaf and stem tissue accounted for about 8% of the osmolality. Previous analyses indicated that the osmotic contribution of other inorganic cations was not significant. If the anion balancing K^+ was assumed to be monovalent, then it was possible to account for approximately 60% of the osmolality.

DISCUSSION

The results presented here show that apoplast solute concentrations are high in nonelongating portions of growing sugarcane leaves and that the concentration of these solutes is higher in younger tissue near the elongation zone. Even in fully mature leaf tissue $\Psi\pi a$ averaged about −0.25 MPa. The presence of these solutes in the apoplast manifests itself as a lowered Ψ in otherwise fully hydrated nontranspiring tissue. These measurements of $\Psi\pi a$ and lowered Ψ nonelongating tissue were not influenced by complicating factors such as the growth-induced water uptake which occurs in elongating tissue. The nature and origin of growth-induced water potentials and lowered Ψ in otherwise fully hydrated tissue is controversial. Cosgrove and Cleland (7) reported a range of $\Psi\pi a$ or young pea and soybean stems similar to that observed for leaf tissue in the present study and concluded that this was the basis for the observed growth-induced Ψ. However, a recent report by Nonami and Boyer (18) concludes that $\Psi\pi a$ in growing pea and soybean stems is actually on the order of only −0.03 to −0.04 MPa and that the principal component of the growth-induced Ψ was negative pressure or tension on the apoplast solution. Some of the results and observations discussed below are relevant to this controversy.

Several factors lead us to conclude that the significant $\Psi\pi a$ measured in nonelongating portions of sugarcane leaves was not an artifact of the measurement and sample handling procedures used. First, as predicted for fully hydrated, nongrowing, nontranspiring tissue, values of bulk tissue Ψ agreed closely with the $\Psi\pi$ of apoplast solution extracted by centrifugation. It should be pointed out that since the tissue samples were not vacuum or pressure infiltrated with water, no correction of these $\Psi\pi a$ values was necessary. Also, avoidance of infiltration treatments minimized alterations in the apoplast environment. Neither the lowered Ψ nor the $\Psi\pi a$ was an artifact due to dehydration during sample handling, since pressure chamber measurements for similar tissue samples invariably yielded balance pressures less than 0.02 MPa. While not a direct measure of $\Psi\pi a$, the $\Psi\pi$ of sap obtained at lower pressures in the pressure exudation experiments was of the same magnitude as $\Psi\pi$ of solution extracted by centrifugation (*cf.* Figs. 4 and 5). Furthermore, in parallel with solution extracted by centrifugation, solute concentration was lower in exudate obtained from older tissue (Fig. 5).

Table III. *Characterization of Solutes in Apoplast Solution Extracted From Sugarcane Leaf Spindle Segments by Centrifugation*

Solution was extracted from segments between 20 and 30 cm from the shoot apex.

Solute	Osmolality	% of Total
	mOsmol kg^{-1}	
K	22	18
Reducing sugars	5	4
Sucrose	11	9
Organic acids	11	9
Unidentified	76	61
Total	125	

On a few occasions exudate in the form of guttation water was collected from margins of intact leaves prior to sunrise in the greenhouse. The $\Psi\pi$ of this exudate averaged −0.03 MPa. Nonami and Boyer (18) obtained similar values for $\Psi\pi$ of exudate collected from the surface of growing pea and soybean stems by applying pressure to the roots submerged in water and concluded that their values represented $\Psi\pi a$. However, for leaves it is questionable whether the $\Psi\pi$ of guttation water reflects $\Psi\pi a$. The sites of exudation of guttation water on leaves are usually in close proximity to the ends of veins (10). Water moving through the xylem to the leaf surface as a result of root pressure could thus bypass most of the apoplastic space. The method used in the present study would appear to provide a more representative estimate of $\Psi\pi a$ because, during centrifugation, solution should be preferentially extracted from the space surrounding living cells. Water in the xylem should be less extractable by centrifugation due to capillary forces and the presence of vessel ends.

Additional indirect evidence for a significant $\Psi\pi a$ can be derived from the comparison of methods used to estimate R_a. If R_a estimated from pressure-volume curves is assumed to be uninfluenced by $\Psi\pi a$, then dividing bulk tissue $\Psi\pi$ by symplastic $\Psi\pi$ (Eq. 1) would yield a lower estimate of R_a than the pressure-volume method if $\Psi\pi a$ were significant. This pattern can be seen in Table I. It also follows that if the magnitude of $\Psi\pi a$ were overestimated then R_a estimated from Eq. 3 would exceed R_a derived from pressure-volume curves. The close agreement between R_a estimated from Eq. 3 and from pressure-volume curves suggests that the values of $\Psi\pi a$ were reasonable (Table I). Finally, symplastic solute content calculated from pressure-volume curve data was invariably lower than bulk tissue solute content calculated from $\Psi\pi$ of sap from frozen and thawed tissue (data not shown), implying that a significant portion of the total tissue solute was located in the apoplast.

Water potentials measured in the elongating zone of sugarcane leaves (Fig. 2) were similar to those reported for elongating maize leaf tissue (23). In contrast to maize leaves, however, Ψ of sugarcane leaves remained substantially lowered in mature leaf tissue. This was verified with *in situ* psychrometry (data not presented). Elongating sugarcane leaf tissue proved to be too delicate to withstand the full complement of measurement procedures applied to nonelongating tissue in the present study. Nevertheless, several lines of evidence make it seem likely that the lowering of Ψ associated with growth in sugarcane leaves is due largely to $\Psi\pi a$. First, the decline in $\Psi\pi a$ as the elongation zone is approached parallels the continuous decline in Ψ to a minimum coinciding with the region of maximum elongation (*cf.* Figs. 2 and 4). While tissue damage precluded direct measurement of $\Psi\pi$ for apoplast solution extracted from elongating spindle segments by centrifugation, it seems unlikely that apoplast solute concentration would abruptly decrease to insignificance inside the elongation zone. On the other hand, it is possible that solute uptake to meet requirements for osmotic maintenance and continued water uptake for cell expansion could diminish apoplast solute concentration to some extent.

Under the conditions described above another component of apoplast Ψ, probably a tension or negative pressure (18) would have to assume greater importance in order to maintain the lowered Ψ measured in the elongation zone. The following observations suggest that this may have occurred. When elongating segments were centrifuged, the filter paper disks in contact with them failed to saturate at the same gravitational force applied to nongrowing segments. Application of greater force damaged elongating segments (19). In the pressure exudation experiments with elongating spindle segments, a threshold pressure between 0.1 and 0.2 MPa was usually required to obtain enough exudate to saturate filter paper disks.

The preceding observations suggest that water in elongating tissue may have been held under a significant tension. However, other observations described below suggest that a negative pressure probably was not the most important component of apoplast Ψ in elongating tissue. Water potential of spindle segments from the zone of maximum elongation exhibited a 0.25 MPa decline during the holding period in the psychrometer chamber (Fig. 3). If this decline is taken to represent a reduction in turgor due to stress relaxation of cell walls (5, 6, 9), then turgor 30 min after excision exceeded the wall yield threshold by at least 0.25 MPa.

It is possible from our data to obtain a preliminary estimate of the yield threshold for elongating sugarcane leaf tissue if tissue Ψ after 9 h (Fig. 3) is taken to be a plateau value (-0.68 MPa) beyond which no significant additional stress relaxation would have occurred. Symplast $\Psi\pi$ (~ -0.92 MPa, Table II) was subtracted from this plateau value of Ψ resulting in an estimated yield threshold of 0.24 MPa. It should be stressed that symplast $\Psi\pi$ was not measured directly in elongating tissue, but was estimated from the average symplast $\Psi\pi$ for tissue 12 to 48 cm from the shoot apex (Table II). Also, this estimate does not take into account changes in $\Psi\pi$ that may have occurred in the psychrometer chamber. Nevertheless, despite these and other potentially confounding factors (9) our estimated yield threshold for sugarcane leaf growth falls within the range of 0.2 to 0.4 MPa reported for other plant tissues (6). These indications that preexcision turgor was at least 0.25 MPa above the yield threshold imply that rate of leaf elongation was more limited by cell wall yielding properties than by hydraulic restrictions on water transport (5, 6) and argues against a negative pressure in the apoplast as the major component of growth-induced Ψ in sugarcane leaves.

The results reported here and by others (2, 7, 11, 14) suggest that a dynamic equilibrium exists between symplastic and apoplastic solutes. Evidence for this can be seen in the pressure exudation experiments (Fig. 5). As expected, the osmolality of the exudate decreased with increasing pressure reflecting the increasing contribution of membrane-filtered symplastic water to the exudate volume. However, osmotic concentration of the exudate obtained above about 0.6 MPa showed no additional decline, suggesting that even the membrane-filtered water contained significant amounts of solutes. The leaf cells were thus rather leaky and did not behave as ideal osmometers. This apparent leakiness decreased with increasing tissue age and was in parallel with the decrease in apoplast solute concentration with increasing age (*cf.* Figs 4 and 5). The effects of 'aging' young spindle segments for 24 h in some experiments, and interrupting exudation for 30 min in others (Fig. 5) strongly suggest that the release of solutes was not an abnormal condition, but represented behavior involved in the maintenance of a tissue age-dependent equilibrium between symplastic and apoplastic solutes. The patterns of solute efflux observed in these experiments may have been due in part to changes in the solute reflection coefficient of cell membranes during tissue maturation. The relatively stable values of osmolality above 0.6 MPa applied pressure should not be interpreted as the absolute values of these equilibrium points, however, since exudate osmolality will depend on rate of exudation and the time interval over which exudate is collected.

Additional work is necessary to partition apoplast Ψ in elongating portions of sugarcane leaves between its osmotic and pressure components and to evaluate the degree to which tissue water transport *versus* cell wall yielding properites limit growth rate. *In situ* measurements of tissue Ψ and turgor, better characterization of the cell wall yield threshold, and a suitable method for extraction of apoplast solution would be helpful in achieving this goal. More complete identification of the apoplast solutes would probably provide some clues concerning their function.

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